bodies. A photodiode measures the amplitude of the wing strokes, and the fly's attempts to turn in one direction or another are proportional to the difference between the two wingbeat amplitudes. The authors first confirmed that flies readily steer away from a focus of expansion, but then they go on to show that flies will steer towards the focus of expansion if the velocity of expanding motion is sufficiently low. Both of these stimuli were presented passively to the fly, but what happens when the fly is allowed to choose the stimulus in front of her? To test this, they turned the flight arena from a passive 'movie screen' into an active 'video game' by coupling the steering signal from the fly's wings to the position of the focus of expansion on the screen, effectively letting the fly decide whether it wanted to either fly towards the focus of expansion by keeping it in front, or avoid that signal and instead fly towards the focus of contraction. In this scenario, flies do indeed fixate the focus of expansion if the velocity of continuous expansion is low, as would be encountered by flying through a distant landscape; however, they rapidly switch from focus of expansion fixation to focus of expansion avoidance if the effective expansion velocity is suddenly increased, as would be encountered by a nearby looming wall.

How does the fly's brain determine whether to fly towards or away from the focus of expansion? Does this behavioral switch require some high-level brain function of the variety seen in primates [7], or can a simpler calculation explain the behavior? Reiser and Dickinson present some computer simulations revealing that a classical model of motion detection, proposed decades ago by Hassenstein and Reichardt [8] and known as the elementary motion detector, is adequate to explain the fly's behavior in tethered and free flight. The proposed control circuit is impressive in its simplicity. The elementary motion detector model is truly 'elementary' in that it computes visual motion by using only two light sensors, delaying the signal of one, then multiplying the two signals. The model thus provides the strongest output when light moves from the delayed to the undelayed sensor. A downstream collating cell would pool the inputs from the many elementary motion detector modules and report the direction of movement over a large visual field. The velocity range that the unit can detect is determined by highpass and lowpass filters, neural computations that are performed on the output of the two sensors.

What is powerful about Reiser and Dickinson's [1] results is that the response of the elementary motion detector model, combined with a simple threshold above which the attraction response switches to an avoidance response, can predict the behavior of flies in free flight without requiring the fly brain to calculate parameters such as the distance to the wall, the time to collision, or even the velocity of the visual expansion. Freely-behaving flies generally avoid flying close to the walls of an arena [9], and an elementary motion detector model with an appropriate lowpass filter and a simple threshold will result in the same behavior, in which the fly turns to avoid the visual expansion once the output of the elementary motion detector model exceeds a particular threshold. The mechanism by which this threshold is set, how and where it is implemented by the brain, and whether or not it is innately fixed or plastic are important open questions.

This new paper [1] completes a triptych of research projects in which three conditions that would very reasonably be encountered during normal forward flight — a gentle headwind, a salient object in the frontal field of view, or relatively slow expansion velocity — are each found to be sufficient to override the powerful expansion avoidance reflex, and instead stabilize flight into an expanding flow field. Reiser and Dickinson [1] show that the simplest known motion detection model, requiring very few processing steps and minimal computational resources, can be used to balance the fly's behavior so that it can progress safely through the world, while at the same time dodging your swatter.

References

1. Reiser, M.B., and Dickinson, M.H. (2013). Visual motion speed determines a behavioral switch from forward flight to expansion avoidance in Drosophila. J. Exp. Biol. *216*, 719–732.
2. Fotowat, H., Harrison, R.R., and Gabbiani, F. (2011). Multiplexing of motor information in the discharge of a collision detecting neuron during escape behaviors. Neuron *69*, 147–158.
3. Card, G., and Dickinson, M.H. (2008). Visually mediated motor planning in the escape response of *Drosophila*. Curr. Biol. *18*, 1300–1307.
4. Tammero, L.F., Frye, M.A., and Dickinson, M.H. (2004). Spatial organization of visuomotor reflexes in Drosophila. J. Exp. Biol. *207*, 113–122.
5. Budick, S.A., Reiser, M.B., and Dickinson, M.H. (2007). The role of visual and mechanosensory cues in structuring forward flight in *Drosophila melanogaster*. J. Exp. Biol. *210*, 4092–4103.
6. Reiser, M.B., and Dickinson, M.H. (2010). *Drosophila* fly straight by fixating objects in the face of expanding optic flow. J. Exp. Biol. *213*, 1771–1781.
7. Mineault, P.J., Khawaja, F.A., Butts, D.A., and Pack, C.C. (2012). Hierarchical processing of complex motion along the primate dorsal visual pathway. Proc. Natl. Acad. Sci. USA *109*, E972–E980.
8. Hassenstein, B., and Reichardt, W. (1956). Systemtheoretische Analyse Der Zeit, Reihenfolgen Und Vorzeichenauswertung Bei Der Bewegungsperzeption Des Russelkafers Chlorophanus. Z. Naturforsch. *11*, 513–524.
9. Tammero, L.F., and Dickinson, M.H. (2002). The influence of visual landscape on the free flight behavior of the fruit fly *Drosophila melanogaster*. J. Exp. Biol. *205*, 327–343.

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Mitochondrial Fission: Rings around the Organelle

Mitochondria form a dynamic network in which organelles fuse or divide in response to metabolic changes or cellular stress. New work shows that mitochondria do not divide in isolation from other cellular structures. Rather, they carry out this process in partnership with the endoplasmic reticulum and actin filaments.

Liza A. Pon

Mitochondrial division (or fission) is mediated by the dynamin-related protein Drp1 and its yeast homologue Dnm1p. Drp1/Dnm1p is a GTPase that is recruited to mitochondria by mitochondrial

outer membrane proteins (Fis1p, Caf4p and Mdv1p in yeast, and Mff in metazoans), and assembles into cylindrical spirals that encircle the organelle. Upon GTP hydrolysis, Drp1/Dnm1p undergoes conformational changes that lead to contraction of the spirals and mitochondrial fission [1]. While a central role for Drp1/Dnm1p in mitochondrial fission is well established, it is clear that Drp1/Dnm1p is not the sole mediator of mitochondrial fragmentation. Specifically, structural analysis indicates that the diameter of the Drp1 ring (30–50 nm) or the Dnm1p ring (100–130 nm) is smaller than the diameter of the mitochondrion [2–4]. Thus, some other pre-constriction factor may act before Drp1/Dnm1p assembly. Recent findings from Korobova *et al.* [5] now raise the very interesting possibility that the endoplasmic reticulum (ER) and actin assemble into a force-generating element that works in conjunction with Drp1 to drive mitochondrial fission.

Organelles are discrete subcellular compartments in which unique environments are created for specific biochemical functions. At the same time, organelles are not autonomous: they interact physically and functionally with one another. Interaction of mitochondria with ER is critical for phospholipid biosynthesis, calcium homeostasis and anchorage of mitochondria at specific sites within cells [6–8]. Indeed, Mfn2, a protein that mediates the interaction of mitochondria with ER as well as mitochondrial fusion, is a target for mutation in Charcot-Marie-Tooth disease type IIa, a peripheral neuropathy [9].

Previous studies point to a role for mitochondria–ER interactions in mitochondrial fission [10]. Specifically, electron tomography studies revealed that ER encircles mitochondria at sites where mitochondria are constricted and are associated with fission proteins (Drp1, its yeast orthologue Dnm1p, and Mff, a mitochondrial fission factor). Importantly, early constriction of mitochondria at sites of ER contact does not require Mff or Drp1. These observations support the idea that ER interacts with mitochondria at sites where mitochondria undergo early constriction events, and that Drp1/Dnm1p is recruited to those sites, where it mediates further constriction of the organelle.

Other studies support a role for actin in mitochondrial constriction. Specifically, treatment of mammalian cells with agents that inhibit mitochondrial electron transport or ATP production results in Drp1-dependent fragmentation of the organelle. Furthermore, disruption of actin inhibits recruitment of Drp1 to mitochondria and attenuates inhibitor-induced mitochondrial fission [11]. These findings support the model that Drp1 serves as a metabolic sensor that alters mitochondrial morphology in response to changes in the oxidative phosphorylation activity of the organelle. They also support a role for the actin cytoskeleton in this process, in part by recruitment of Drp1 to the organelle. However, the mechanism underlying actin function in mitochondrial fission was not well understood.

The new work from the Higgs laboratory now reports evidence for a direct role for actin and a formin protein in mitochondrial fission [5]. Formins are conserved proteins that regulate the dynamics of actin and microtubule cytoskeletons [12]. INF2 is an ‘inverted’ formin: its formin homology domains (FH1 and FH2) are closer to the amino terminus of the protein compared with other formins. This inverted formin stimulates actin nucleation and elongation of F-actin, like other formins. In addition, it stimulates F-actin depolymerization at filament pointed ends. There are two INF2 isoforms in mammalian cells: one is bound to ER through its CAAX box and regulates ER morphology [13]; the other lacks a CAAX box and is found in cytosolic actin meshworks but also stabilizes the Golgi apparatus [14].

Korobova *et al.* [5] find that actin localizes to sites of ER–mitochondria interaction in mammalian cell lines. Moreover, they obtained evidence that INF2 stimulates actin polymerization at sites of mitochondrial fission, and that this actin polymerization is required for recruitment of Drp1 to those sites. Specifically, they find that silencing of the ER-associated INF2 results in elongation of mitochondria and defects in both assembly of Drp1p into punctate structures and association of Drp1 with mitochondria. Consistent with this, they show that overexpression of constitutively active ER-associated INF2 has the opposite effect, resulting in mitochondrial fragmentation that is dependent upon Drp1p and the actin polymerization activity of INF2.

These data support a model for ER, actin and formin function in mitochondrial fission (Figure 1). According to this model, ER and its associated INF2 encircle mitochondria at sites of mitochondrial fission. INF2 then stimulates actin polymerization at that site. Actin can generate forces by different mechanisms, including myosin-mediated filament sliding and polymerization-driven pushing forces. Therefore, it is likely that actin provides the force for constriction of mitochondria to a diameter that is compatible with the size of the Drp1p cylinder. This then allows for the assembly of Drp1 into spirals and cylinders at that site and a second round of constriction that ultimately leads to mitochondrial fission.

This study reveals a novel mitochondrial–cytoskeletal interaction, an ER- and formin-dependent mechanism for establishing that interaction, and a foundation for understanding how this interaction affects mitochondrial dynamics. It also raises questions regarding the precise function of actin in mitochondrial fission. Does actin serve as a scaffold or force generator that allows ER to encircle mitochondria? Alternatively, is actin required for generating forces for constriction of mitochondria? Indeed, these models are not mutually exclusive. If actin generates forces for mitochondrial constriction, what is the mechanism underlying this process? Does actin assemble into a contractile ring, similar to the actomyosin ring that mediates cytokinesis? Alternatively, does newly polymerized actin that extends from INF2 on the ER surface

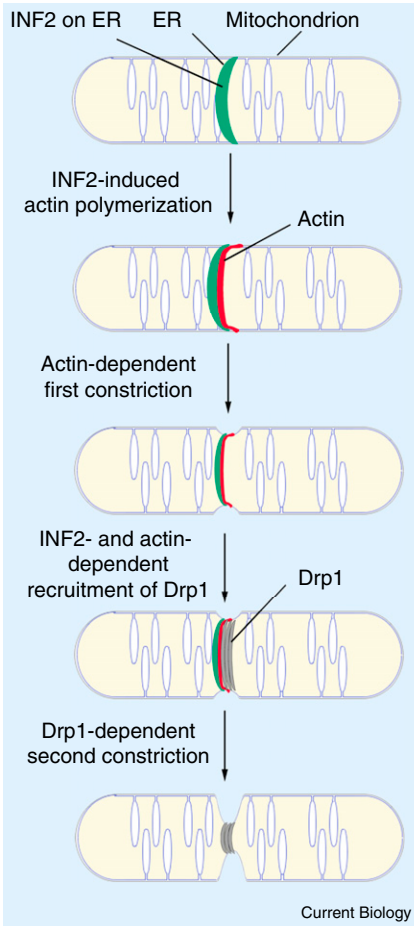

Figure 1. Model for mitochondrial fission.

ER encircles mitochondria at sites of mitochondrial fission. INF2 that is associated with ER then stimulates polymerization of actin, which provides the force for partial constriction of the organelle. Drp1 assembles into spirals and cylinders at the constricted site, where it drives more constriction of the organelle and mitochondrial fission.

exert inward pushing forces on mitochondria?

How INF2 is regulated and how it contributes to constriction of mitochondria is yet to be determined. Mutagenesis studies indicate that INF2 is regulated by autoinhibition like other formins and that actin polymerization by INF2 is required for its function in mitochondrial fission [5]. Thus, INF2 is likely activated at sites of mitochondrial fission. Moreover, INF2 is unique among formins because it stimulates actin depolymerization as well as polymerization. Is there an INF2 activator on mitochondria? What controls the length of F-actin that is polymerized at sites of mitochondrial fission? Is the actin depolymerization activity of INF2 also required for its function in mitochondrial fission?

These studies also raise questions regarding the mechanism for recruitment of Drp1 and Mff to ER-marked sites of mitochondrial fission. Does Mff bind to actin? Or does it recognize mitochondrial membrane curvature either directly or by binding to a protein that recognizes that curvature?

Finally, can budding yeast, in which actin is intimately associated with mitochondria and ER, shed light on this process in other eukaryotes? Neither of the known formins in budding yeast localizes to ER [12]. However, Bni1p is found in the cytosol, and could, like INF2, stimulate actin polymerization at sites of ER–mitochondrial contact [15]. Other studies revealed a protein complex (mitochore/ERMES) that is required for association of mitochondria with the actin cytoskeleton and for mitochondrial morphology and motility [16]. Interestingly, this complex also mediates the association of mitochondria with ER [17]. Thus, it is possible that mitochore/ERMES maintains actin at sites of mitochondria–ER interactions, which in turn contributes to mitochondrial fission through effects on mitochondrial constriction and recruitment of dynamin-related proteins to those sites.

Future studies that address these fundamental questions will provide a foundation for understanding the interaction of mitochondria with ER and the actin cytoskeleton, mechanisms that underlie and regulate fission of the organelle, and forces that control mitochondrial plasticity. INF2 is a target for mutation in a degenerative kidney disease (focal and segmental glomerulosclerosis) and a peripheral neuropathy (Charcot-Marie-Tooth disease). Therefore, these studies will also extend our understanding of the role of mitochondrial fission in human disease.

References

1. Elgass, K., Pakay, J., Ryan, M.T., and Palmer, C.S. (2013). Recent advances into the understanding of mitochondrial fission. Biochim. Biophys. Acta *1833*, 150–161.
2. Smirnova, E., Griparic, L., Shurland, D.L., and van der Bliek, A.M. (2001). Dynamin-related protein Drp1 is required for mitochondrial division in mammalian cells. Mol. Biol. Cell *12*, 2245–2256.
3. Ingerman, E., Perkins, E.M., Marino, M., Mears, J.A., McCaffery, J.M., Hinshaw, J.E., and Nunnari, J. (2005). Dnm1 forms spirals that are structurally tailored to fit mitochondria. J. Cell Biol. *170*, 1021–1027.
4. Mears, J.A., Lackner, L.L., Fang, S., Ingerman, E., Nunnari, J., and Hinshaw, J.E. (2011). Conformational changes in Dnm1 support a contractile mechanism for mitochondrial fission. Nat. Struct. Mol. Biol. *18*, 20–26.
5. Korobova, F., Ramabhadran, V., and Higgs, H.N. (2013). An actin-dependent step in mitochondrial fission mediated by the ER-associated formin INF2. Science *339*, 464–467.
6. Swayne, T.C., Zhou, C., Boldogh, I.R., Charalel, J.K., McFaline-Figueroa, J.R., Thoms, S., Yang, C., Leung, G., McInnes, J., Erdmann, R., *et al*. (2011). Role for cER and Mmr1p in anchorage of mitochondria at sites of polarized surface growth in budding yeast. Curr. Biol. *21*, 1994–1999.
7. Rowland, A.A., and Voeltz, G.K. (2012). Endoplasmic reticulum-mitochondria contacts: function of the junction. Nat. Rev. Mol. Cell Biol. *13*, 607–625.
8. Lackner, L.L., Ping, H., Graef, M., Murley, A., and Nunnari, J. (2013). Endoplasmic reticulum-associated mitochondria-cortex tether functions in the distribution and inheritance of mitochondria. Proc. Natl. Acad. Sci. USA *110*, E458–E467.
9. de Brito, O.M., and Scorrano, L. (2008). Mitofusin 2 tethers endoplasmic reticulum to mitochondria. Nature *456*, 605–610.
10. Friedman, J.R., Lackner, L.L., West, M., DiBenedetto, J.R., Nunnari, J., and Voeltz, G.K. (2011). ER tubules mark sites of mitochondrial division. Science *334*, 358–362.
11. De Vos, K.J., Allan, V.J., Grierson, A.J., and Sheetz, M.P. (2005). Mitochondrial function and actin regulate dynamin-related protein 1-dependent mitochondrial fission. Curr. Biol. *15*, 678–683.
12. Chesarone, M.A., DuPage, A.G., and Goode, B.L. (2010). Unleashing formins to remodel the actin and microtubule cytoskeletons. Nat. Rev. Mol. Cell Biol. *11*, 62–74.
13. Chhabra, E.S., Ramabhadran, V., Gerber, S.A., and Higgs, H.N. (2009). INF2 is an endoplasmic reticulum-associated formin protein. J. Cell Sci. *122*, 1430–1440.
14. Ramabhadran, V., Korobova, F., Rahme, G.J., and Higgs, H.N. (2011). Splice variant-specific cellular function of the formin INF2 in maintenance of Golgi architecture. Mol. Biol. Cell *22*, 4822–4833.
15. Buttery, S.M., Yoshida, S., and Pellman, D. (2007). Yeast formins Bni1 and Bnr1 utilize different modes of cortical interaction during the assembly of actin cables. Mol. Biol. Cell *18*, 1826–1838.
16. Boldogh, I.R., Nowakowski, D.W., Yang, H.C., Chung, H., Karmon, S., Royes, P., and Pon, L.A. (2003). A protein complex containing Mdm10p, Mdm12p, and Mmm1p links mitochondrial membranes and DNA to the cytoskeleton-based segregation machinery. Mol. Biol. Cell *14*, 4618–4627.
17. Kornmann, B., Currie, E., Collins, S.R., Schuldiner, M., Nunnari, J., Weissman, J.S., and Walter, P. (2009). An ER-mitochondria tethering complex revealed by a synthetic biology screen. Science *325*, 477–481.

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